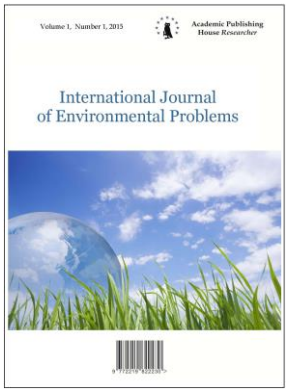
Published in the Slovak Republic
International Journal of Environmental Problems
Has been issued since 2015.
E-ISSN: 2413-7561
2018, 4(1): 24-28

DOI: 10.13187/ijep.2018.1.24
www.ejournal33.com



Bioactivity of Crude Extracts of Stem Bark of *Vetillariaparadoxa*

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Abstract

This research aimed to study the bioactivity of crude extracts of stem bark of *Vetillariaparadoxa* "Shea-nut tree" as used in traditional medicine for treatment of stomach ache and control of diarrhea, using hexane, acetone and ethanol as the extraction solvents. Phytochemical screening of stem bark of extracts of *Vitellariaparadoxa* revealed the presence of alkaloids, steroids, phenolic compounds, flavonoids, saponins, tannins, and cardiac glycosides. Ethanol, acetone, and hexane extracts inhibited the growth of pathogenic *Escherichia coli, salmonella typhi,* and *staphilycoccusaureus* with varying degrees of activity with the ethanol extract demonstrating the highest activity against all the tested bacterial organisms.

Keywords: phytochemicals, antimicrobial, secondary metabolites, bioactivity, inhibition.

1. Introduction

Vitellariaparadoxa (Sapotaceae) is a plant that is locally abundant in Nigeria in the derived Savannah zones, particularly near towns and villages. It is rich in oil and replaces the oil palm as a source of edible oil in Northern Nigeria (Njoku, 2011; Borokini, 2014). The plant species *(Vitellaria)* is easily distinguished by its very long leaf stalks, more widely spaced nerves and abundant white latex when slashed and in the petiole. Shea-butter is the fat extracted from the kernel of this plant. It is becoming increasingly popular as a component of cosmetic formulation in addition to its long standing use as a cocoa butter substitute in the chocolate industry (Omwirhiren et al., 2016).

Shea butter contains high level of UV-absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols (Leke, 2015). Research have confirmed that she a butter contains a high level of unsaponifiables (5 – 15 %) which include: campesterol, stigmasterol, sitosterol, spinosterol and triterpenes such as cinnamic acid ester and amyrin, parkeol, butyrospermol. lupeoland and a hydrocarbon called karitene. Analysis of the kernel reveals the presence of phenolic compounds such asgallic acid, catechin, epicatechin, epicateachingallate, gallocatechin, epigallocatechin, epigallocatehingallate, as well as quercetin and trans-cinnamic acid (Borokini, 2014; Leke, 2015).

V. paradoxa (formally *Butryspermumparadoxum*), *(Sa-potaceae)* is an immensely popular tree with many applications in folkloric medicine. It is commonly called Shea butter (English), Kareje (Fulfulde, Nigeria), Kadanya (Hausa, Nigeria) Koita (Gbagi, Nigeria), mameng (Cham, Nigeria). The tree grows naturally in the wild of the dry savanna belt of West Africa, from Senegal

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in the West to Sudan in the East and onto the foothills of the Ethiopian mountains. V. paradoxais considered a sacred tree by many communities and ethnic groups and plays important roles in religious and cultural ceremonies. It is also believed to have some spiritual protective powers (Eleazu et al., 2012; Rajeshwar et al., 2016). Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections (Rajeshwar et al., 2016; Eltayeb et al., 2018). The bark is used to suppress cough and also to treat leprosy (Patil, Gaikwad, 2011; Eltayeb et al., 2016).

The research aims to assess the phytochemicals and antibacterial activities of the plant using three solvents and to compare same.

2. Materials and Methods

Preparation of reagents

Mayer's Reagent

Dissolve 1.36 g of$HgCl_2$ and 5 g of KI in distilled water separately, mix both solutions and make it up to 100 ml with distilled water.

Wagner's test (Iodo-potassium iodide)

Dissolve 2 g of iodine and 6 g of KI in 100 mL of distilled water.

Dragendorff's reagent

Stock solution-Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few min with 50 ml glacial acetic acid. After 12h, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. To the clear, reddish brown filtrate (40 ml) 160 ml ethyl acetate and 1 ml distilled water was added and stored in amber–colored bottle.

Working Solution-To the 910 ml of stock solution, 20 ml of acetic acid was added and made up to 100 ml with distilled water.

Sample collection and preparation

Fresh stem bark of *Vitellariaparadoxa* (2.5 Kg) were collected from Janligo village of Yana, Shira L.G.A in Bauchi State, Nigeria. The plant was identified by a local medical practitioner Mr.KabiruAdamu (Dan-Chakwati) from Shira L.G.A Bauchi State and authenticated by a Botanist. The Sample was dried under (shade) room temperature for over 21 days and grinded using mortar and pestle, sieved in order to obtain a pure powdery form. The powdered material was stored in polythene bags at room temperature until needed.

Extraction

50 g of the dried and pulverized fiber-free stem bark of *Vitellariaparadoxa* was extracted exhaustively via maceration with 3·300cm^3 of hexane (HE), acetone(ACE) and ethanol(EE), each at room temperature for 24 h. Excess solvent was removed to give crude extracts of HE, ACE, and EE, from hexane, acetone and ethanol respectively.

Phytochemical Screening

Crude hexane extract (HE) was screened for phytochemicals using standard procedures (Harborne, 1973; Sofowora, 1993).

Phytochemicals screening methods

Detection for Alkaloids, flavonoids, tannins, phenolic compounds, tannins, saponins, terpenoids, sterols and glycosides were done using standard procedures.

Thin layer chromatography of *V. paradoxa Crude* Extract

Three chromatography papers of the same length were used. Three different mixtures of solvent system varying polarities were also used to enhance ideal separation in different chromatography tanks: hexane/chloroform (1:1), chloroform, chloroform/ethyl acetate (1:1) and ethyl acetate/ethanol (1:1).

The solvent front and the separation were later calculated and the relation factor was obtained using the formula.

$R_f = m^1/m$

Were R_f – relation factor. m^1 – separations (cm). m – solvent front (cm).

The disc diffusion method

The paper disc diffusion assay technique (Akpuaka et al., 2003) was done.

Preparation of the medium

The nutrient agar medium was prepared by dissolving 7.0 g of agar in 250 ml of distilled water in a conical flask and heated to dissolve. The solution was sterilized in an autoclave at 121°C for 15 min, cooled and poured into Petri dishes to set.

Preparation of culture media and inoculation

Culture of *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*were obtained from the microbiology laboratory of Abubakar Tafawa Balewa University, Bauchi, Nigeria. Pure isolates were obtained by sub-culturing unto fresh nutrient agar plates. The bacteria were separately used to inoculate the Petri dishes. The plates were incubated at 37 ± 2°C for 24 h.

Assay of extracts

Two different concentrations of each extract were obtained by suspending 250 mg of each extract in 6.0 ml of absolute ethanol and the volume was made up to 10.0 ml using sterile distilled water to give a concentration of 25 mg/ml. These also served as stock solutions. The second concentrations were obtained by diluting 2.0 ml each of the stock solutions with 2.0 ml of sterile distilled water giving a concentration of 12.5 mg/ml. Sterile 6 mm Whatman's filter paper discs were soaked in the extracts and placed on the plates and incubated for 24 h at 37 ± 2°C. The plates were examined for clear zones of activity. The zones of inhibition were measured using a transparent plastic meter ruler

Broth diffusion method

Equal volumes (1.0 ml) each of the extract solutions were mixed with same volume (1.0 ml) of an overnight broth culture of *S. aureus* and *E. coli* to give solutions of final concentrations of 12.5 and 6.25 mg/ml, respectively, in a test tube. These were then incubated at 37 ± 2°C for 24 h and observed for the presence of bacterial growth using a compound microscope

Inoculation and application of extracts

Bacterial strains were inoculated in 15 ml of sterile nutrient broth an inoculated at 37°C for 24 h then the 3 different extracts 0.5ml was introduced to the plates and left for 24 h.

Minimum Inhibitory Concentration (MIC)

This is the lowest drug concentration that prevents visible microorganisms' growth after overnight incubation, a plate of solid growth media. After a pure culture is isolated was examined and minimum inhibitory concentration was determined.

3. Results

Table 1. Weight of various macerated fractions of *Vetillariaparadoxa*

Fraction	Observation	Weight (g)
V. paradoxa + Ethanol	Reddish brown	2.60
V. paradoxa + Acetone	Dark brown	3.70
V. paradoxa + N-hexane	Yellow brown	1.70

Table 2. Result of phytochemical screening

Phytocompounds	Regents	Extracts		
		ACE	HE	EE
Alkaloid	Dragendroff's Mayer's	+ +	+ +	- -
Flavonoids	1% ammonia, 2m NaOH, + HCl	++ ++	+ +	- -
Tannins	5% $FeCl_3$	+	-	+
Saponins	Olive Oil	+	+	-
Terpenoids	Salkowski	++	+	-

Glycosides	Legal's Kelarkillani	+ +	- -	+ +
Steroids	Salkowski	+	-	+
Phenols	1% gelatin solution 10% NaCl	+	+	+

+ Slightly Present; ++ Moderately Present; +++ Highly Present; – Absent

Table 3. Antibacterial activity and zone of inhibition (mm) of *V. paradoxa* disc diffusion

Extracts	Concentration, mg/ml	*E. Coli*	*S. Typhi*	*S. Aureous*
EE	25.0 12.5	6 -	8 -	10 -
ACE	25.0 12.5	7 -	7 -	2 -
HE	25.0 12.5	1 -	2 -	3 -
Streptomycin		25	NT	NT

NT – not tested/done; – no inhibition

Table 4. Various fractions from thin layer chromatography of stem bark of *V. paradoxa*

Extracts	Solvent System	Retention Faction (R.F)
HE	hexane/chloroform chloroform/ethyl acetate ethyl acetate/ethanol	0.6 0.3 0.5
ACE	hexane/chloroform chloroform/ethyl acetate ethyl acetate/ethanol	0.7 0.8 0.8
EE	hexane/chloroform chloroform/ethyl acetate ethyl acetate/ethanol	0.5 0.4 0.3

4. Conclusion

Phytochemical screening of hexane, acetone, and ethanol, extracts revealed the presence of flavonoids, tannins, terpenoids, saponins, sterols, alkaloids and cardiac glycosides by positive reaction with the respective test reagents. Phytochemical screening showed that maximum presence of secondary metabolites was in acetone extract than in the hexane, and ethanol, where almost all the phytocompounds appeared in the acetone extract (Table 1), whereas tannins, glycosides, and steroids where absent in hexane extract; alkaloids, flavonoids and saponins were absent in the ethanol extract. Antimicrobial susceptibility of the extracts (50 mg/ml) against the test organisms showed that in all the three solvents used, the ethanol extracts demonstrated the highest activity, followed by acetone, whereas hexane demonstrated the least activity on the tested bacteria as seen in (Table 3).

Preliminary phytochemical investigations of stem bark of *Vitellariaparadoxa* revealed the presence of some/many secondary metabolites. These secondary metabolites are linked to microbial activity of the plant material and the extracts of this plant has antimicrobial effects on the tested enteric bacteria, hence serve as potential therapeutic agents against diarrhea and other microbial afflictions.

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